DEVELOPMENT OF SOME SPECIES OF PHOLIOTA

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(WITH PLATES XVI–XX)

The taxonomy of the Agaricaceae at the present time is based upon characters of the mature plant which in many cases are slight and superficial and of uncertain homology. It is very probable that a knowledge of the origin and method of development of the different structures composing the mature fruit body would aid greatly in determining true relationships among the different genera and species, and it is for this reason that a comparative study of the morphology and development of the basidiocarp is important.

The first serious study in the Agaricaceae of the origin and differentiation of the parts of the young fruit body began over half a century ago, when HOFFMANN (19), in 1856, briefly described the origin of the hymenium in *Agaricus campestris* and two other species. Four years later HOFFMANN (20) gave a brief account of the development of several additional species, in all of which the hymenium was exogenous in origin except one, *Marasmius oreades*.

In 1866 DEBARY (13) studied the development of several species of Agaricaceae, and his work was followed in 1874 by HARTIG'S (18) description of *Armillaria mellea*, BREFELD'S (12) work upon *Coprinus* in 1877, and in 1889 by FAYOD'S (15) very cursory study of 43 species, with hymenium both exogenous and endogenous in origin. Nothing more was done along this line of research until 1906, when ATKINSON (2) published a thorough description of the development of *Agaricus campestris*, and the stimulus given by this work has been evidenced during the last 10 years by several publications on the development of different agarics, notably those of ALLEN (1), BEER (11), and FISCHER (16).

The material for the following investigation was collected during the late summer of 1915 in the vicinity of Seventh Lake, in the Adirondack Mountains, New York. The 3 species, *Pholiota squarrosa*, *P. flammans*, and *P. adiposa*, were all growing upon

rotten coniferous wood, presumably *Picea rubra*. Material of all 3 species was very abundant in several different localities, and in each case the young stages selected were identified beyond the possibility of a doubt by mature specimens associated with them. An abundance of material in all stages of growth was fixed in Carnoy's fluid and carried into cedar oil before returning to Ithaca, where it was imbedded, some in paraffin and some in collodion, and sectioned for study.

Pholiota squarrosa

Young fruit bodies, in the stage of development shown in fig. 1, are elliptical or elongate in outline and composed of hyphae loosely interwoven in the basal region, but more compact toward the apex, with some of the threads radiating from the summit. Scattered through the tissue of the fruit bodies are hyphal threads, somewhat straighter and more even in diameter than the ordinary hyphae, which are conspicuous because of their property of taking a very deep stain. These differentiated hyphae are present not only in the youngest basidiocarps, but in successive stages of development, and in all 3 species studied. Their function is unknown, but probably they serve some special purpose in nutrition. Glycogen has been shown to occur in large quantities in many fungi (*Phallus*, et al.) as a reserve food material, utilized during growth, and it may be that these peculiar threads owe their deep-staining properties to the presence of this substance.

DIFFERENTIATION OF STEM FUNDAMENT.—In the fruit body shown in fig. 1 a small, deeply staining area occurs in the central apical part; this is a region of active growth, with hyphae slightly more slender and richer in protoplasm than the other hyphae of the fruit body. This region marks the apex of the stem fundament, and in younger stages of development than the one represented here would in all probability be found to originate in the base of the fruit body, as shown in fig. 25 for *P. flammans*. In this species, as will be described more fully later, the fundament of the stem appears first in the base of the fruit body as a deeply staining area, which by progressive growth and differentiation moves to the apical part (fig. 26). A similar origin of the stem fundament has been

described in *Lepiota cristata* and *L. seminuda* (10), in species of *Cortinarius* (14), and in *Rozites gongylophora* (22).

In further stages of development, the hyphae in the stem fundament, by interstitial growth, form a compact, broadly conical area, whose apex is the dark-stained region and whose sides in median longitudinal sections slope outward at a strong angle (fig. 8). The hyphae pursue a rather uniformly longitudinal direction of growth, and are rich in protoplasm; the peripheral threads, because of this longitudinal arrangement and their deeply staining qualities, delimit the surface of the stem from the enveloping ground tissue, whose hyphae are poor in protoplasmic content and without definite direction.

DIFFERENTIATION OF HYMENOPHORE AND PILEUS PRIMORDIA.—During an early stage in the differentiation of the stem fundament there appears, in median longitudinal sections, in the ground tissue on either side of the apical part of the fundament, a small mass of hyphae, which is readily distinguishable from the surrounding tissue because of the compact nature of the hyphal complex and its property of taking a deep stain (figs. 4, 7). Serial longitudinal sections show that these hyphae occur in a ring around the apex of the stem primordium; they are the earliest evidence of the differentiation of the primordium of the hymenophore. The appearance of the hymenophore primordium differentiates the fundament of the pileus from the stem fundament, although as yet the tissue composing it is very loose and hardly to be distinguished from the surrounding ground tissue. The individual hyphae that make up the hymenophore primordium grow down from this area; at first they are crowded, very rich in protoplasm, and run in every direction, or in some cases may even be wound up into a ball (fig. 6). As the development of the fruit body proceeds, the hyphae grow out of the tangled condition in which they exist in the youngest stages of the hymenophore primordium and take a downward course. By the intercalary growth of new hyphae from above the primordium of the hymenophore becomes more compact and broadens out, keeping pace with the margin of the pileus primordium, which by interstitial and marginal growth is continually moving centrifugally toward the periphery of the fruit body. At first the hyphal threads

in the hymenophore are slender and somewhat pointed; they show a tendency to aggregate themselves at the tips into groups or tufts, with the ends of several hyphae in each tuft, and the different tufts separated by narrow interstices, so that the primordium often presents a rough and jagged appearance in this stage of its development.

BLEMATOGEN.—In the youngest fruit body sectioned (fig. 1) the universal veil consists of hyphae which push up at the apex and turn downward in all directions. There is little doubt that if younger stages had been available for study, a condition would have been found similar to that in the very young fruit bodies of *P. flammans*, where the hyphae in the beginning are loose and radiate from all over the surface of the basidiocarp (fig. 24). In the stage shown here, however, the development has proceeded to a condition where the hyphae of the lateral surface of the fruit body have taken on a direction of growth parallel to the axis of the stem fundament; a central core or strand of hyphal threads in the apex grow upward more rapidly than the surrounding tissue, and by curving backward and downward form a covering, which is the blematogen, over the entire surface of the fruit body. The hyphal cells thus exposed upon the outside become enlarged and many of the outermost ones lose their protoplasmic content and become oval or globose. The hyphae composing the fruit bodies shown in fig. 1 are 3–5 μ in diameter near the base, and in the apical region they are slightly more slender, averaging about 3 μ in thickness, while the hyphae of the blematogen layer are much thicker, being 8–15 μ in diameter. The condition existing here is somewhat similar to the origin of the blematogen in *Coprinus lagopus* (12), as described and figured by BREFELD. In this plant, according to BREFELD, before pileus formation, the fruit body consists simply of the stem primordium, whose outer hyphae have free ends, and do not enter into the formation of the stem; the pileus primordium is differentiated at the apex "upon the point of the stem," and from this pileus primordium hyphae grow out and turn downward in all directions, forming the "pileus-volva." In the meantime the loose peripheral threads of the stem primordium, the "stem-volva," grow upward to meet these downward growing hyphae from the

apex, and the two groups of threads grow into each other and intermingle to form the common mass of the "volva." In *P. squarrosa* there is a material difference from the condition just described for *C. lagopus*, since in this case there are no hyphae on the stem surface which grow upward and unite with the downward growing threads. Figs. 2, 3, and 7 show the central strand of hyphae just mentioned; in fig. 5 the character of the blematogen hyphae may be seen.

ORGANIZATION OF PILEUS.—Coincident with an early stage in the development of the hymenophore primordium, median longitudinal sections show that the fundament of the pileus is becoming differentiated from the surrounding tissue (fig. 8). The hyphae become richer in protoplasm and by interstitial growth form a more compact structure. This organization proceeds from the center outward in a centrifugal manner, the margin of the pileus keeping pace with, and contributing to, the growth of the hymenophore primordium. During the early stages of differentiation of the pileus some of the hyphae arise from the stem, but its later growth is probably due entirely to interstitial and marginal increase of its own elements, which are interwoven in all directions, thus differing from the hyphae of the stem, which in general run parallel to the stem axis. The pileus elements merge gradually with the blematogen and there is no sharp line of division between the two structures. The cells of the blematogen hyphae, however, are swollen and have thick walls, which stain deeply, while the pileus hyphae are slender and do not take a deep stain after the pileus is well organized, so that a general distinction is evident. In addition, the peripheral threads of the pileus, composing the "cortex," are very compact and form a dark line between the pileus and blematogen, as shown in fig. 13.

FORMATION OF PALISADE LAYER.—Following the stage when the hyphae are arranged in irregular tufts, the hymenophore primordium becomes more compact (fig. 12). The ends of the individual hyphae grow down to nearly the same level and become clavate. This even area is the palisade layer. Serial sections show that it is formed first around the stem, while the hymenophore near the pileus margin is still in the uneven, jagged stage. The

pileus margin at this time is turned downward and often somewhat incurved as a result of epinastic growth, and lies nearly parallel with the surface of the stem, which still slopes outward at a slight angle (fig. 10).

FORMATION OF ANNULAR PRELAMELLAR CAVITY.—During differentiation of the young basidiocarp some ground tissue is left below the hymenophore primordium in the angle formed by the junction of the stem and pileus fundaments. In later development this ground tissue increases to some extent by interstitial growth, but the more rapid growth of the stem, hymenophore, and pileus subjects it to tension, and it very early becomes loose in texture (fig. 4). As the stem elongates and the pileus broadens out, this tension is further increased, so that the ground tissue becomes still looser, with large spaces between the hyphae. At first it only partially tears away from the surface of the hymenophore, and as a result the gill cavity thus formed is weak, with strands of ground tissue traversing it (figs. 13, 14, 16–20). The strength of the prelamellar cavity varies in different individuals, as has been shown to be the case in *Agaricus rodmani* (8); but in any case, in later stages, but long before the gills are exposed by rupture of the veil, the strands of ground tissue become completely broken away, and the edges of the lamellae are entirely free within the gill cavity.

ORGANIZATION OF PARTIAL VEIL.—The terms "blematogen" or "universal veil" and "marginal" or "partial veil" have been interpreted by ATKINSON (5), and are used in the same sense here. The formation of the blematogen has already been described. The radial growth of hyphae in the apex of the young fruit body is very rapid for a time, and a thick layer is formed, enveloping the entire plant, but it is more dense in the apical region (figs 7–9). This rapid growth soon ceases, however, the hyphae become thick-walled and poor in protoplasmic content, the peripheral ones die, and become enlarged and swollen. Because its growth has nearly ceased, the universal veil becomes subject to tension from the expansion of the parts within and breaks up into the large, conspicuous, squarrose scales (fig. 13) which cover the stem and pileus and give the plant its specific name. The partial veil has its origin in

the ground tissue left in the angle between the stem and hymenophore. This tissue increases, both by interstitial growth and by the addition of hyphae which grow down from the pileus margin (fig. 22). By the time that the gills are well formed this tissue occupies a considerable area lying between the margin of the pileus and the surface of the stem, and forming the floor of the gill cavity. It is covered externally by the blematogen, with the inner surface of which its hyphae are interlaced, as some of them are with the stem surface. When expansion of the pileus occurs and the veil is ruptured, it is left upon the stem as an annulate membrane composed of two layers, the coarse, scaly blematogen layer below and the partial veil above.

Origin and development of lamellae.—In a recent publication Atkinson (9) has shown that in the Agaricaceae thus far studied there are two types in regard to the origin and development of the lamellae. First, the "*Agaricus*" type, in which the gills arise by downward growing radial salients of the hymenophore, accompanied or preceded by a more or less well developed annular prelamellar cavity. Second, the "*Amanita*" type, in which there is no general annular prelamellar cavity, and the origin of the lamellae is a series of trabeculae extending from the pileus fundament to the stem, and attached to both. *P. squarrosa* obviously belongs to the first type of development, since we have already seen that in the course of development of the young fruit body a general annular, prelamellar cavity, though weak, and a palisade layer are formed.

The origin and differentiation of the gills from the hymenophore is centrifugal; the later formed and younger parts are nearer the margin, becoming progressively older as the stem is approached. Successive stages in the origin and development of the lamellae, therefore, may be studied by means of serial longitudinal sections, commencing at the pileus margin and going toward the center of the fruit body.

Figs. 16–21 represent such a series of sections. The basidiocarp from which they were made was one selected from material imbedded in collodion, and the sections, 15–20 μ thick, were cut with the sliding microtome. The use of collodion (or celloidin) as

an imbedding material obviates the difficulty sometimes met with in the use of paraffin, that delicate structures may be deformed or dislocated by the heat of the oven or in spreading the paraffin ribbons. Furthermore, the cutting of thicker sections, with a sliding stroke, offers little chance for the displacement of structures, which might happen in cutting thin paraffin sections. I mention these points because some might suspect that the tearing away of the ground tissue below the hymenophore, as shown in the following figures, might be due to manipulation of the tissue, but such is not the case.

Fig. 16 represents a section near the margin of the pileus; the hyphae of the hymenophore are growing down in little tufts, and at this time present a very loose, uneven surface. A considerable number of hyphae from the ground tissue below may be seen spanning the prelamellar cavity and united indiscriminately with the downward growing tufts of the hymenophore and with the hyphae in the spaces between them.

In fig. 17, from a section a little nearer the stem, the hymenophore on either side of the section presents the same loose, uneven surface as in the preceding figure; but in the middle the hyphae have enlarged at the tips and become blunt, and the ends have grown down to form an even surface, the palisade, from which the ground tissue is almost entirely broken away. The reason that the palisade is in the middle of the section, with undifferentiated tissue on either side, is readily seen, if we remember that the hymenophore is in the form of a circle around the stem apex, so that tangential sections, in passing toward the center, cut through older parts of the hymenophore in the middle of the section, with younger parts on either side. For further details see the papers on *Agaricus rodmani* and *Coprinus* species by ATKINSON (**8**, **9**), where, by means of diagrams, the orientation of the parts of the fruit body in relation to longitudinal sections is made very clear.

In fig. 18, in addition to the undifferentiated tissue on either side and the palisade layer, two slightly downward projecting folds of the latter may be seen. These slight downward projections of the palisade layer represent the first origin of the lamellae in this area. To one a strand of ground tissue is attached, while the other

is completely free. That this ground tissue has nothing to do with the formation of the palisade is shown by the fact that, as already stated, it is attached indifferently to the hymenophore primordium, and in many cases is largely broken away from the tufts and between them before the palisade layer is formed. Furthermore, these tufts of the hymenophore primordium are not the primordia of the lamellae, since before the origin of the latter they become lost in the even palisade (figs. 17, 18). In those instances where the ground tissue remains adherent to the edges of the lamellae for some time, and not to the palisade between them, it is due to the fact that through the downward growth of the gills the strands of hyphae attached to their edges are subject to less strain than the hyphae attached between them, and so keep their attachment longer.

The downward growth of the lamellae may be partly initiated by the pressure in the palisade layer due to the rapid growth and enlargement of its hyphae, which would produce a tendency to throw the palisade into folds. The chief agency in their formation, however, seems to be the downward growth of radially arranged groups of hyphae in the hymenophore, which are very active in growth at this time, as indicated by their deep stain. These radial lines of deeply staining hyphae push down into the folds of the palisade and form the trama of the gills (figs. 19–21). The further growth of the lamellae in depth takes place by apical and interstitial growth in the trama. Later stages in development (fig. 23) show the hyphae from the trama turning outward on all sides to add to the growth of the palisade layer of the gills.

Pholiota flammans

PRIMORDIUM OF BASIDIOCARP.—The very young fruit body, before any internal differentiation has taken place, is a compact structure, composed of slender, intricately interwoven hyphae, 2–3 μ in diameter, and rich in protoplasm. The hyphae have a general direction of growth away from the substratum, and many free hyphal ends radiate outward all over the surface of the basidiocarp, so that the peripheral area is somewhat looser in structure than the compact central region (fig. 24). This loose radiating zone of hyphae upon the outside represents an early stage in the

development of the blematogen, which is probably present from the first appearance of the fruit body primordium.

DIFFERENTIATION OF STEM FUNDAMENT.—As development proceeds, the hyphae in the base of the primordium take on more active growth than the others, and by interstitial increase form a very dense structure. This new area of growth, which is the stem fundament, is shown as a deeply stained region in the base of the fruit body in fig. 25. As growth continues, the cone-shaped stem fundament advances toward the apex of the fruit body. In fig. 26 the most deeply stained portion represents the rapidly growing, progressive apex of the stem fundament; the more compact tissue below represents its earlier differentiated base; and the outer zone of loose tissue surrounding the whole is the blematogen.

DIFFERENTIATION OF HYMENOPHORE AND PILEUS PRIMORDIA.—The first evidence of the hymenophore primordium is the appearance of a ring of compact, slender hyphae which surrounds the upper part of the stem fundament and grows down into the ground tissue, clothing the latter. The appearance of these differentiated hyphae marks off the pileus area from the stem fundament. In some cases the pileus fundament probably exists before the appearance of the hymenophore primordium, as indicated by the slight divergence of the hyphae from the apex of the stem fundament or by the more rapid growth in the region of the future pileus; but no sharp distinction can be drawn between the fundaments of pileus and stipe until the primordium of the hymenophore is differentiated. An early stage in the development of the latter is shown in fig. 30. As development continues, the pileus and hymenophore progress together in growth in a centrifugal manner. New elements from the pileus margin contribute to the hymenophore primordium, which also increases by interstitial growth. Interstitial growth also takes place in the pileus primordium, making it more compact. In the stage of figs. 31 and 32 the stem and pileus are well organized and the hymenophore has become compact, with uneven surface. The palisade is formed later, as shown in figs. 34 and 37. The hyphae are not as crowded, or so conspicuously clavate, as in *P. squarrosa*.

BLEMATOGEN.—The blematogen is present from the first appearance of the fruit body primordium. During early development

it radiates from all parts of the surface as a loose aggregation of hyphae with numerous interhyphal spaces (fig. 29). The new elements arise chiefly in the apex of the young fruit body and extend outward in a radial direction, curving backward as the fundament of the basidiocarp elongates and as the stem and pileus primordia are differentiated. This peculiarity of the blematogen is like that in *P. squarrosa.* This downward growth continues until a thick covering is formed (fig. 31). The elements of the blematogen are thick-walled and somewhat larger than the other hyphae of the basidiocarp, but they are not conspicuously globose, as in *P. squarrosa.*

Early in its formation some of the threads begin to break down and gelatinize, and the universal veil soon becomes a gelatinous matrix, imbedded in which may be recognized the remnants of hyphae not yet disorganized. Such a condition exists in fig. 31, and a high magnification (fig. 38) shows a sharp contrast between the gelatinized blematogen and the cortex of the pileus at this stage. A very similar condition of the universal veil has been noted in *Stropharia ambigua* by ZELLER (**25**).

The disorganization of the blematogen elements does not go beyond the degree shown in fig. 38. Sections perpendicular to the pileus in mature specimens show a very similar condition; there is a gelatinous ground substance filled with dead hyphae whose general course is parallel to the surface of the pileus. Through drying of the outer part and the tension exerted upon it by the expanding pileus the blematogen breaks up into scales. These scales differ very markedly, however, from the stout, pointed scales of *P. squarrosa;* they are thin and delicate and more or less fibrillose in nature.

The gelatinization takes place only over the pileus, the blematogen clothing the stem very nearly resembling that of *P. squarrosa.* For this reason the scales covering the pileus in the mature plant are more delicate than those covering the stem, as indicated in the description of *P. flammans* in SACCARDO'S *Sylloge* (**23**), in which it is stated that the pileus is covered with superficial scales, while the stem is scaly-squarrose.

FORMATION OF ANNULAR GILL CAVITY AND MARGINAL VEIL.—The formation of the gill cavity agrees in all essentials with its

formation in the preceding species. The ground tissue is loose from a very early stage, and through expansion of the different parts of the fruit body it becomes torn away from the lower surface of the hymenophore. This separation from the hymenophore is completed at an earlier stage than in *P. squarrosa*, and consequently a well defined cavity is present before the origin of the lamellae (figs. 34, 37).

During the development of the hymenophore primordium and the breaking away of the ground tissue below hyphae are growing down from the pileus margin. These threads penetrate the ground tissue below the prelamellar cavity and mingle with those on the surface of the stem. In fig. 33 they may be seen curving inward from the pileus margin. In the stage represented here they have not yet reached the surface of the stem, and the loose ground tissue surrounding the latter may still be seen between it and the advancing hyphae from the pileus margin. These threads are sharply contrasted with the other tissue of the basidiocarp because of their short cells, which in some cases are as broad as they are long. In addition, they may be distinguished from the blematogen external to them by their deeper stain. These hyphae from the pileus margin, together with the ground tissue below the hymenophore, form the partial veil; it tears away from the stem at an early stage in the expansion of the plant, and may in some cases hang down from the pileus margin as a thin appendiculate veil.

ORIGIN AND DEVELOPMENT OF LAMELLAE.—The origin and development of the gills in this plant does not differ materially from the condition already described for *P. squarrosa*. Here we have a better defined gill cavity, with no ground tissue traversing it and connecting with the palisade layer. The latter is composed of hyphae with blunt ends, standing in an even surface, compact, but not crowded. Serial longitudinal sections, from the tangential to the median, show that the formation of the annular gill cavity, of the hymenophore, and the development of the lamellae, all proceed by centrifugal growth; that is, the older and first formed parts of these structures lie near the stem and progress by differentiation and growth toward the periphery of the fruit body. As has already

been stated, the hyphae in the palisade layer are not crowded enough to produce any great pressure, and it would seem that the origin of the gill salients, which appear as downward folds of the palisade, is due entirely to the growth and elongation of radial lines of hyphae in the hymenophore, which push the palisade down in folds, the young gills, as described for *Hypholoma sublateritium* (1) and *Stropharia ambigua* (25). The gill salients are broader than in *P. squarrosa*, and this may be due to the fact that when thrown into folds by downward growth of the hyphae above, because of the less crowded condition of the palisade, they are not subjected to as great lateral pressure as in that species.

Fig. 39 is from a tangential longitudinal section of a basidiocarp with sterile gills, that is, the palisade layer has failed to form. The cystidia, which develop from the trama of the gill, are very noticeable as deeply staining clavate bodies. The situation presented here is interesting because of its bearing on the question recently raised by LEVINE (21) in regard to the origin of the lamellae in the Agaricaceae. The points of growth for the origin of the lamellae, as described by ATKINSON in several species of the Agaricaceae, including *Agaricus rodmani* (8) and *Coprinus comatus*, *C. atramentarius*, and *C. micaceus* (9), occur in radial areas of hyphae in the hymenophore, which develop centrifugally and grow down more rapidly than the other hyphae. These areas are the gill tramae, and push the palisade into regularly spaced folds, which are the salients of the lamellae themselves. This method of origin of the lamellae occurs in the three species described here.

According to LEVINE'S conception, radiating ridges of palisade cells arise in the fundamental tissue, and by continued differentiation and downward growth of new palisade cells, split apart. The adjacent halves of neighboring ridges then come together and unite to form a lamella. The trama of the gill would thus be formed by the coming together of preexisting palisade cells from adjacent ridges. If no palisade cells were differentiated, therefore, no trama could be formed. In this case, however, no palisade cells are formed, yet the trama of the gill and the cystidia, which come from trama tissue, develop normally.

Pholiota adiposa

PRIMORDIUM OF BASIDIOCARP.—In the youngest fruit body sectioned the mycelial threads grow out from the substratum to form a compact mass of hyphae which are closely interwoven and run in all directions. From this structure hyphae gradually assume an upward direction of growth, forming a papilla-like projection (fig. 42), the fruit body primordium. The threads in the basal mycelium from which the fruit body arises are very uneven in size; those of the primordium are even in size, with free ends radiating out all over the surface.

STEM FUNDAMENT.—The stem fundament probably differentiates first in the base of the fruit body, as in the preceding species. In fig. 43 its apex appears as a compact, dark-staining region near the top of the basidiocarp, surrounded by the looser tissue of the young blematogen. The fundament hyphae are very slender at first, dense in protoplasm, and closely intermingled, running in all directions.

PRIMORDIA OF HYMENOPHORE AND PILEUS.—When the hymenophore and pileus primordia appear, the stem has become well organized as a compact conical area, surrounded by the loose universal veil. The first differentiation of the pileus fundament becomes evident through the growth of hyphae upward from the stem apex; these spread outward laterally, so that at this stage the stem and pileus areas together resemble a sheaf of wheat. At the same time some of these hyphae become subject to strong epinastic growth, and curve down in a ring around the stem apex, forming the primordium of the hymenophore, which definitely differentiates the pileus area from the stem fundament. The growth of the pileus continues by interstitial and marginal increase of its elements, and the hymenophore broadens out by growth within itself and by intercalary growth from the pileus margin, with which it keeps pace. The hyphae of the hymenophore primordium often become aggregated into tufts (fig. 48), as in *P. squarrosa*. Ground tissue may still remain attached to these tufts. The hyphae now grow down to the same general level, their ends become blunt, and form an even surface. During early development the hymenophore extends down on the stem farther than in the two preceding

species (figs. 45, 47). In later stages it loses this decurrent character.

BLEMATOGEN.—The universal veil exists from the beginning; its development proceeds very much as in *P. flammans*. At first its hyphae radiate from all over the surface of the basidiocarp. Later the growth of new elements is largely confined to the apex. The peripheral cells become enlarged, thick-walled, with a diminution in protoplasmic content (fig. 52). The outer ones appear empty and dead. Gelatinization takes place here, as in *P. flammans*, but later, after the gills are well formed. Sections through the mature pileus show that the blematogen has a structure very comparable in the two species, in either case composed of a structureless matrix in which are imbedded dead hyphae, with a general course parallel to the pileus surface. At first disorganization occurs only over the pileus, but in the mature plant the gelatinization takes place over the entire surface.

The mature pileus in *P. flammans* is dry, and in *P. adiposa* is gelatinous or viscid. This difference is due to the fact that in the latter species the disorganization of the blematogen elements proceeds farther than in the former, so that the walls of the hyphae become more gelatinous, with a greater capacity for absorbing water. The surface of the blematogen breaks up into scales, as in *P. flammans*, but the scales are very different in character. They are not thin and fibrillose here, but in wet weather appear like little lumps of jelly on the surface and are easily lost, so that it is not uncommon to find old fruit bodies with the surface of the pileus nearly free from them, especially over the central area.

ANNULAR PRELAMELLAR CAVITY.—The gill cavity begins to develop soon after the appearance of the hymenophore and pileus primordia. The growth and expansion of these parts tear apart the ground tissue below the former, making it loose, with large spaces between the hyphae. At first, strands of hyphae span the cavity and remain attached to the hymenophore, but these have all disappeared by the time the palisade layer is formed, and the cavity is then clear (fig. 47).

MARGINAL VEIL.—The partial veil is composed of the ground tissue left in the angle between the hymenophore and stem, together

with some hyphae which grow down from the pileus margin. This growth, however, is not as strong as in *P. flammans*. The partial veil is covered externally by the blematogen. It ruptures at the pileus margin during expansion of the plant, leaving a thin and fugacious annulus on the stem.

Origin and development of lamellae.—The origin and growth of the gills take place in this species much as in *P. flammans*. The first evidence of the origin of the gill salients is the downward projection of the palisade layer in broad folds (figs. 50, 51). A single one of these broad folds includes several of the tufts which earlier appear in the hymenophore primordium and become lost in the palisade; consequently, these tufts cannot be considered as gill fundaments or directly concerned in their origin. At the apex of the folds the ends of the palisade cells may in some cases spread slightly apart, showing that considerable pressure is exerted by the downward growing hyphae from the hymenophore above. Under no circumstances, however, do the gill salients show any evidence of splitting; the hyphae merely spread slightly apart at the ends, and in later stages come together again to form an uninterrupted palisade. Serial sections show that the formation of the gill salients is radial and centrifugal.

Figs. 53, 54, and 55 show a condition that might easily lead to a wrong interpretation of the origin of the lamellae by one not familiar with the orientation of the parts involved. A similar condition existing in *Agaricus rodmani* has been explained by Atkinson (8) and so will not be gone into in detail here. The sections are tangential in the margin of the pileus at a stage in development when the pileus margin is enrolled. The attachment of the gill trama both above and below does not mean that the trama of the gill has grown down and united with the tissue below, as might appear at first glance. The pileus margin, and consequently the hymenophore, is incurved so that the hymenophore lies both above and below the gill cavity, and the attachment of the trama below as well as above represents its point of origin. The direction of growth of the trama is not in the plane of the section, but perpendicular to it. A similar condition exists in *P. squarrosa* and *P. flammans* at a certain stage in the organization of the pileus before it has expanded.

Sequence of plant parts

The relative time of origin of the primordia of the basidiocarp is of some historical interest. Fries (17), influenced perhaps by the preformation theory, still in vogue in his time, believed that all the parts, pileus, stem, and hymenophore, although indistinguishable, existed already formed in the young fruit body and unfolded simultaneously. Schmitz (24) held that a successive formation of new parts occurred; that the development of new parts rose upward just as gradually as in the higher plants, so that those standing higher came into evidence later than those below; and that therefore the matrix developed before the stipe, the latter before the pileus, and the latter before the hymenium. Later, Fayod (15) formulated a general law to the effect that the first part to be differentiated is always the pileus primordium.

More recent work has shown that no general rule can be laid down as to what primordium shall have precedence in differentiation. In *Agaricus campestris* (2), *A. arvensis* (3), *A. rodmani* (8), *Armillaria mellea* (4), and *Stropharia ambigua* (25), the hymenophore primordium is differentiated first. In *Hypholoma sublateritium* (1), *H. fasciculare* (11), and *Amanitopsis vaginata* (7) the pileus area is first outlined. The formation of the stem fundament is the first differentiation to take place in *Lepiota cristata* and *L. seminuda* (10), several species of *Cortinarius* (14), *Rozites gongylophora* (22), and the 3 species of *Pholiota* described.

Even in the same species variations may occur as to the relative time of appearance of the different primordia. Atkinson (10) has shown this to be probable in *Agaricus arvensis* (3) and *Lepiota clypeolaria* (6). In *P. flammans* it would appear that the fundament of the pileus in some cases differentiates before the hymenophore primordium, and this may be true of the other two species described. In all 3 species, however, the appearance of the stem fundament precedes the other primordia of pileus and hymenophore.

Summary

1. The basidiocarp primordium consists of slender hyphae intricately interwoven; they are arranged more loosely in the

peripheral region, and radiate from the entire surface of the fruit body.

2. The blematogen is present from the first differentiation of the fruit body primordium, and in its earliest stages consists of the loose, radiating peripheral hyphae. In subsequent growth it forms a thick layer enveloping the entire plant; in *P. flammans* and *P. adiposa* it becomes partially disorganized by gelatinization.

3. The formation of the stem fundament is the first differentiation to take place in the young fruit body. It originates in the basal part of the basidiocarp and by growth and differentiation progresses toward the apex.

4. The primordium of the hymenophore is differentiated around the apex of the stem fundament as an annular internal zone of new growth. Frequently, before the hymenophore appears, a slight divergence of hyphae from the stem apex indicates differentiation of the pileus. When the hymenophore primordium is differentiated, it marks off clearly the limit between the pileus and stem. It consists of slender hyphae, rich in protoplasm, which grow downward. At first the lower surface is uneven and loose, but by continued growth the hymenophore becomes compact and the hyphae grow down to the same level, forming an even palisade area. The growth of the hymenophore and organization of the pileus proceed centrifugally.

5. The annular prelamellar cavity is formed by the tearing away of the ground tissue from the lower surface of the hymenophore, due to the tension exerted by the growth and expansion of the plant parts. It is weak in *P. squarrosa*, and well formed in *P. flammans* and *P. adiposa*, before the origin of the lamellae.

6. The marginal veil consists of two layers; the inner portion is composed of the ground tissue left in the angle between the stem and the hymenophore after their differentiation, which increases by its own growth and by the addition to it of hyphae from the pileus margin. The outer portion is a section of the universal veil between the pileus margin and the surface of the stem.

7. The lamellae originate as a series of radiating areas of active hyphae in the hymenophore, which grow down and push the palisade layer into folds. The points of growth for the origin of the gills are

in these downward growing areas in the hymenophore, and the first folds in the palisade are the salients of the lamellae themselves. In *P. flammans* and *P. adiposa* the gill salients are very broad; in all 3 species their origin and differentiation is centrifugal and their subsequent growth is downward into the gill cavity.

In conclusion, I wish to acknowledge my deep obligation to Professor G. F. ATKINSON, under whose direction this work was done, for his unfailing interest and many helpful suggestions.

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EXPLANATION OF PLATES XVI–XX

The following microphotographs were made with the Bausch and Lomb vertical camera and Zeiss lenses, and with a horizontal Zeiss camera.

PLATES XVI, XVII

Pholiota squarrosa

FIG. 1.—Median section, showing apex of stem fundament as dark-stained area near center of figure; very deeply stained hyphae scattered through stem fundament; hyphae of blematogen radiating from summit; $\times 50$.

FIG. 2.—Slightly older stage than above, showing central strand of hyphae that curve downward from summit to form blematogen; $\times 50$.

FIG. 3.—Median section, showing radiation of blematogen hyphae and their loose arrangement at periphery of fruit body; $\times 50$.

FIG. 4.—Median section at a stage closely following differentiation of hymenophore; cone-shaped stem fundament occupies lower part of figure, with hymenophore primordium on either side of deeper stained apex, showing as two small, deeply stained areas, which differentiate pileus primordium above from stem fundament; below hymenophore primordium ground tissue is becoming loose in structure; outside the whole is the thick universal veil; $\times 33$.

FIG. 5.—Left side of same section, magnified more to show character of blematogen; at extreme left are enlarged, thick-walled, empty cells of outer

portion of blematogen; at extreme right may be seen a part of hymenophore primordium with loose ground tissue below; ×56.

FIG. 6.—Left side of median section about the time when hymenophore primordium is first differentiated; tangled, compact mass of hyphae of very young hymenophore near center, with looser ground tissue surrounding it; ×133.

FIG. 7.—Median section at about the same stage of differentiation as fig. 4; two compact, dark spots are hymenophore primordium; from summit blematogen hyphae are curving outward and downward; ×50.

FIG. 8.—Median section; conical stem fundament below; hymenophore primordium on either side, and pileus fundament appearing above hymenophore as a more compact, central area; loose ground tissue clothes stem, and outside is deep-stained blematogen; ×18.

FIG. 9.—Tangential section of same fruit body; hymenophore primordium appears as dark horizontal area, with uneven lower surface; irregular dark region just below is oblique section of stem surface; ×18.

FIG. 10.—Median section, at a stage when palisade has formed; gill cavity appears as a narrow slit almost closed because of epinastic growth of pileus margin; below, the looser ground tissue; ×133.

FIG. 11.—Tangential section of same fruit body; ground tissue is tearing away from hymenophore to form gill cavity; above hymenophore is crescent-shaped pileus, with the much thicker blematogen outside; ×33.

FIG. 12.—Higher magnification of section near preceding to show blunt ends of palisade hyphae, with loose ground tissue below; above is dense hymenophore; ×133.

FIG. 13.—Blematogen has broken into scales; stem and pileus are well organized; below hymenophore on either side is gill cavity, weak, with strands of ground tissue traversing it; dark line between pileus and hymenophore indicates area of more rapid growth; ×13.

FIG. 14.—Left side of a section similar to fig. 13, and showing same features enlarged; ×32.

FIG. 15.—Tangential section, showing compact hymenophore; no gill cavity in this section because its centrifugal development has not yet reached tangential area here shown; ×18.

FIGS. 16–20.—Serial tangential sections from pileus margin toward stem; in fig. 16 hymenophore is uneven and gill cavity is weak, with considerable ground tissue still attached to hymenophore; in fig. 17 hymenophore has become even in center, forming a palisade area; gill cavity is stronger than in preceding section; in fig. 18 two gill salients appear as folds in level palisade; figs. 19 and 20 show older stages of these and other salients nearer stem; ×32.

FIG. 21.—Higher magnification of fig. 20, showing origin of gill tramae in hymenophore as regularly spaced, deeper staining areas of rapidly growing hyphae; ×128.

FIG. 22.—Left side of median section in a stage after lamellae are well differentiated; at extreme left are hyphae growing down from the pileus margin into ground tissue below; knife has passed lengthwise through a gill, making it thin enough to look through and see level palisade between it and next gill; $\times 128$.

FIG. 23.—Tangential section, showing gills with trama and palisade; $\times 148$.

PLATES XVIII, XIX

Pholiota flammans

FIG. 24.—Median section of very young fruit body, which is broken just above substratum of rotten wood; loose, radiating threads represent early stage in formation of blematogen; no internal differentiation has taken place; $\times 60$.

FIG. 25.—Stem fundament forming in base of fruit body, its apex stained very deeply; outside is blematogen; $\times 36$.

FIG. 26.—Slightly later stage in which, by progressive growth, apex of stem fundament has advanced to summit of fruit body; $\times 36$.

FIG. 27.—Somewhat older fruit body, with stem fundament differentiated, apex showing near summit as darker area; very deeply stained, isolated hyphae are scattered through stem fundament; $\times 36$.

FIG. 28.—Same as fig. 27; $\times 36$.

FIG. 29.—Older fruit body, with blematogen radiating from entire surface; stem area well organized, but no other differentiation; $\times 36$.

FIG. 30.—Small area on either side with looser ground tissue below represents very young hymenophore; below is conical stem area and above is pileus fundament which is partially organized; over pileus area blematogen hyphae are spreading downward and have begun to gelatinize; on sides blematogen hyphae are still radiating laterally; $\times 20$.

FIG. 31.—Stem and pileus areas well organized; hymenophore shows as very deeply stained area on either side below pileus; hyphae from pileus margin are shown growing down and uniting with stem surface; outside is lighter stained, partially gelatinized blematogen; $\times 36$.

FIG. 32.—Tangential section of same, showing uneven stage of hymenophore, with ground tissue in gill cavity; $\times 36$

FIG. 33.—Left side of median section; uneven surface of hymenophore primordium is shown above weak gill cavity; outside hymenophore, hyphae from pileus margin are growing in toward stem surface; majority of these hyphae have not reached stem and ground tissue is seen between them and the latter; at extreme left is blematogen; $\times 86$.

FIG. 34.—Right side of median section at a later stage; gill cavity well formed; above it hymenophore has organized palisade near stem, but is still uneven near pileus margin; short-celled hyphae from latter are filling in ground tissue below cavity to help form partial veil; $\times 142$.

FIG. 35.—Tangential section, showing early stage in organization of palisade by hymenophore; gill cavity below; ×125.

FIG. 36.—Tangential section very near stem, showing two broad gill salients, covered by palisade; some clavate cells of latter project beyond surface, giving rough appearance; note disorganized condition of universal veil; ×86.

FIG. 37.—Tangential section, showing even palisade and large gill cavity; ×31.

FIG. 38.—Section highly magnified to show contrast between pileus and gelatinized blematogen; ×142.

FIG. 39.—Tangential section of fruit body with sterile gills; note absence of any palisade, and the cystidia, which develop from trama tissue of gill; ×142.

FIG. 40.—Tangential section, showing normal gills at about same stage as preceding; hyphae of trama may be seen turning outward to contribute to palisade; cystidia are visible on edges of lamellae; ×142.

FIG. 41.—Tracheid of *Picea* inclosed in tissue of stipe; this fruit body was in advanced stage of development, with lamellae well differentiated; ×230.

PLATE XX

Pholiota adiposa

FIG. 42.—Median section of very young fruit body; narrow, dark area in center is a bit of substratum inclosed in tissue; threads radiating from surface form young blematogen; ×50.

FIG. 43.—Later stage, showing apex of stem fundament at summit of basidiocarp; ×56.

FIG. 44.—Still later stage in development; ground tissue is becoming loose around apex of stem fundament; blematogen hyphae have largely ceased lateral growth and are radiating out from summit of fruit body; ×33.

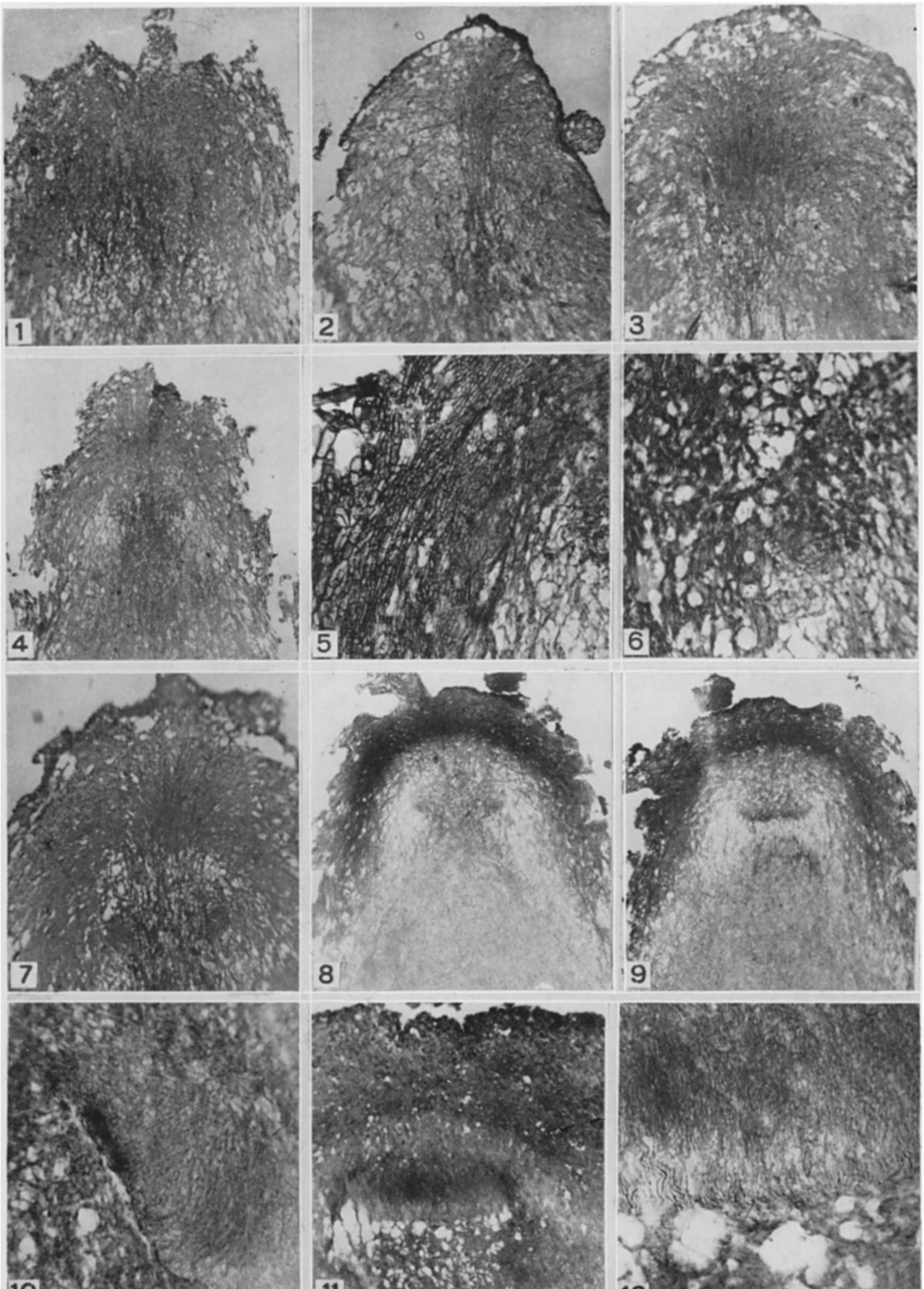
FIG. 45.—Median section of stage when stem and pileus are well organized and hymenophore is forming palisade; heavy blematogen shows no signs of disorganization that occurs later; ×18.

FIG. 46.—Tangential section at same stage in development; because it is nearer pileus margin hymenophore has not formed palisade in this section; ×18.

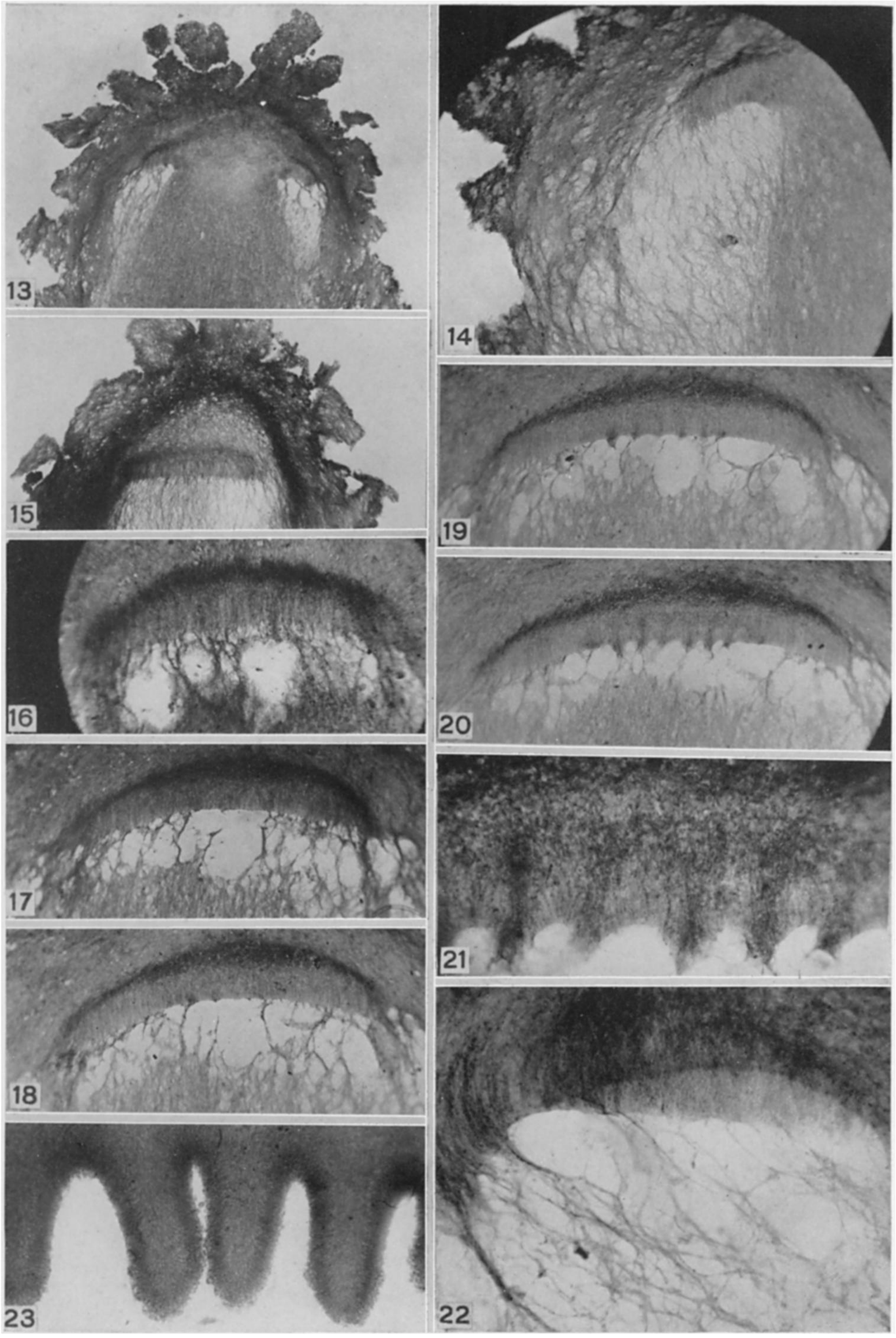
FIG. 47.—Left side of median section; note that hymenophore is decurrent on stem at this stage; gill cavity is clearly differentiated and free from ground tissue; ×154.

FIG. 48.—Tangential section near pileus margin; uneven hyphae of hymenophore primordium are aggregated into tufts; gill cavity is just beginning to form by tearing away of ground tissue from hymenophore; ×154.

FIG. 49.—Section of same fruit body, but nearer stem; palisade forming in middle region of hymenophore surface; ×93.



SAWYER on PHOLIOTA



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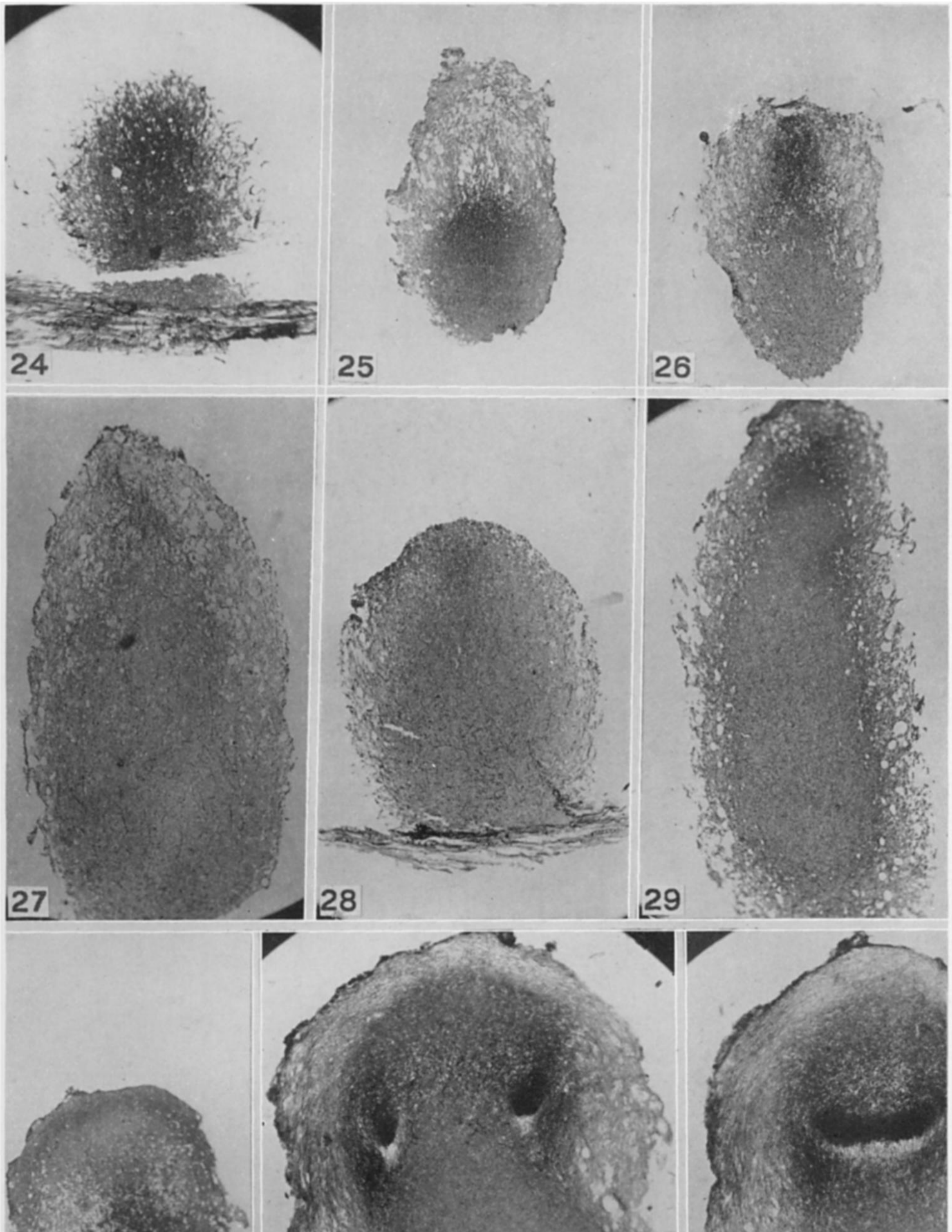

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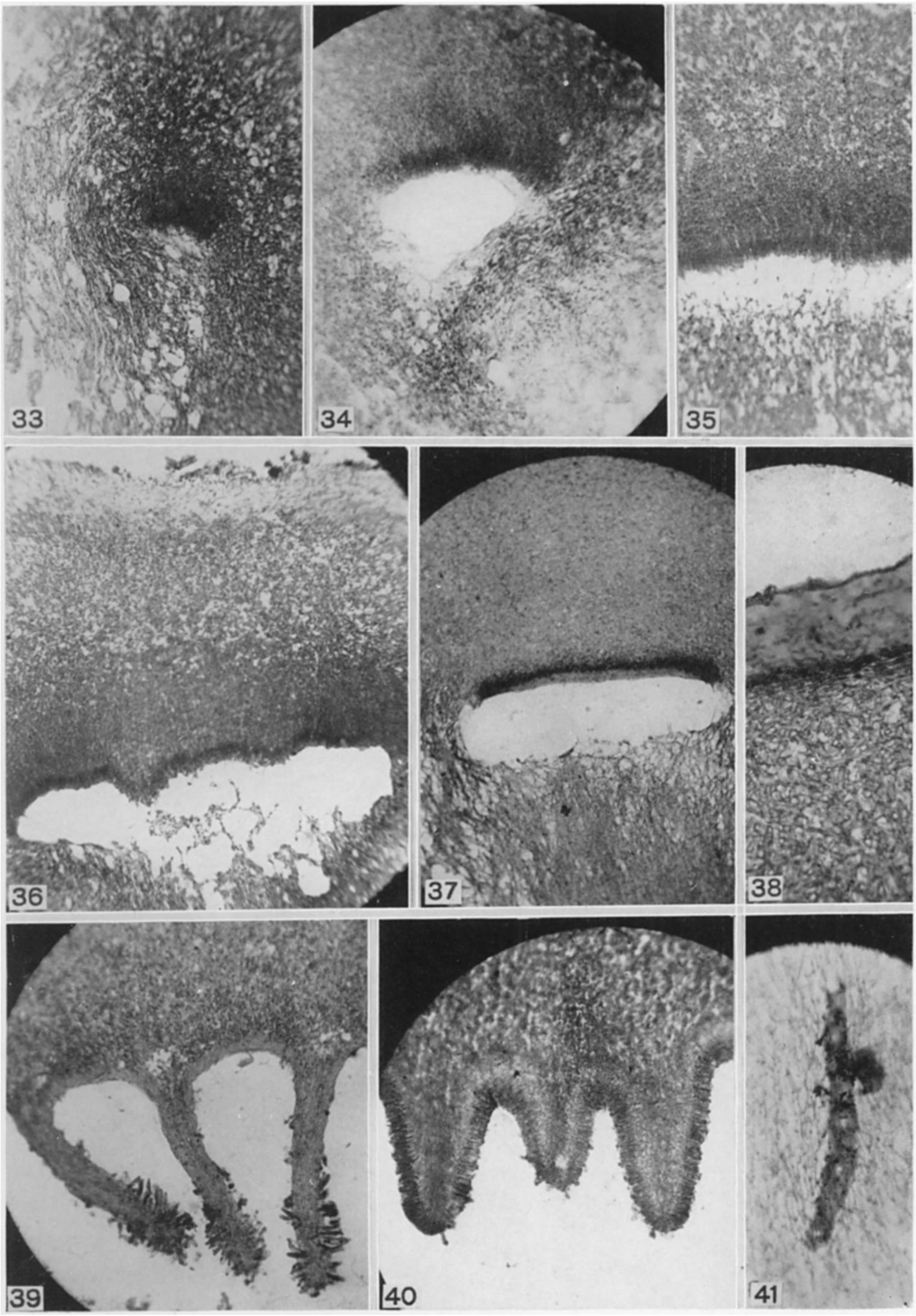

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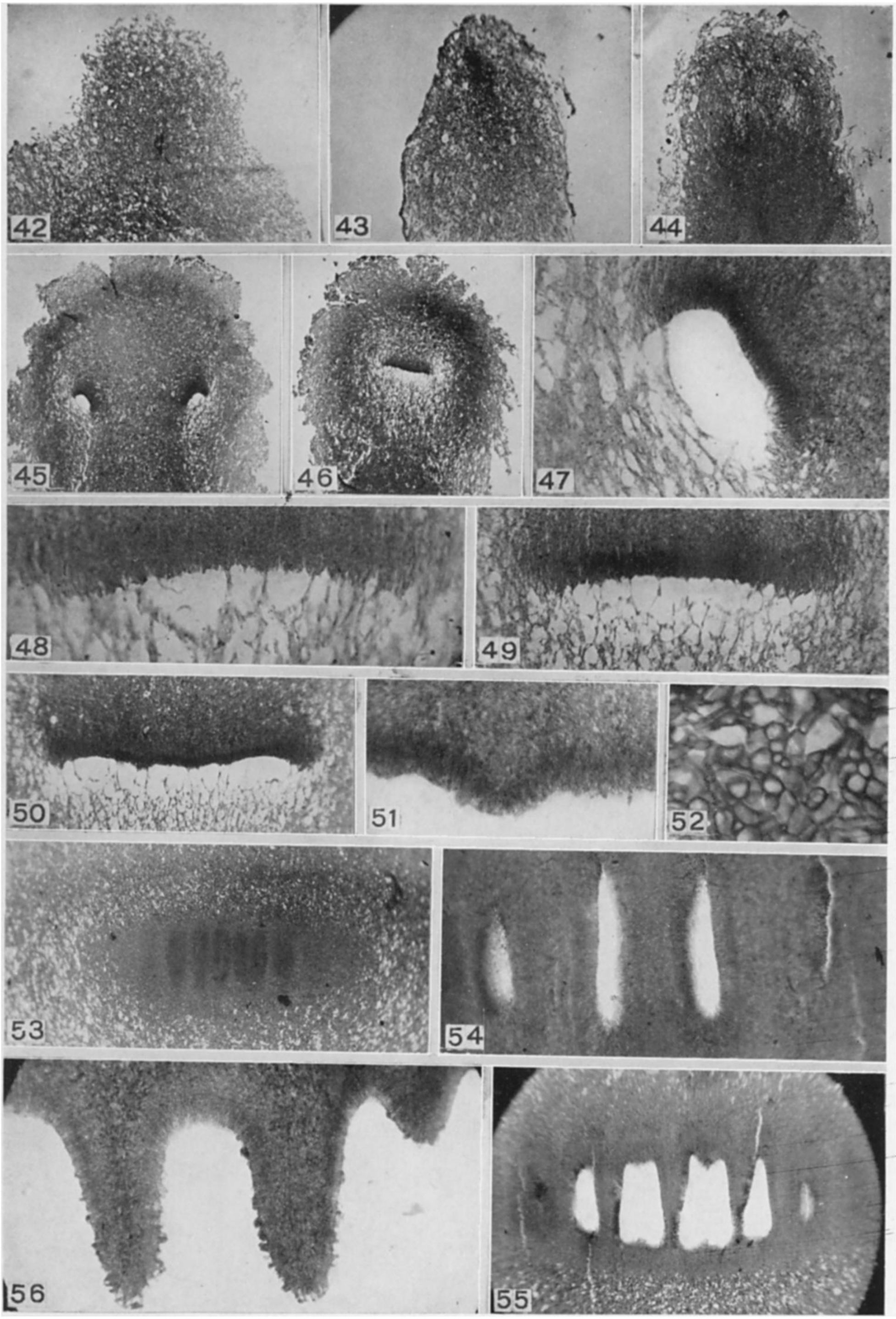

SAWYER on PHOLIOTA

FIG. 50.—Same, nearer stem; note the 3 slight downward projections of palisade, which are very young gill salients; some ground tissue still clings to them because their downward growth relieves tension upon it to some extent; $\times 67$.

FIG. 51.—Section from same fruit body near stem, showing very broad gill salients; $\times 250$

FIG. 52.—Large, thick-walled cells of blematogen; $\times 250$.

FIGS. 53–55.—Sections tangential in enrolled pileus margin; fig. 53 ($\times 33$) has not cut into gill cavity; "backs" of gills show as deeper stained areas; in fig. 54 ($\times 154$) gill cavity is reached and gill is shown in middle with trama in center and palisade on either side; its origin is in hymenophore both above and below; fig. 55 ($\times 56$) is nearer stem.

FIG. 56.—Tangential section, showing well-formed gill, with trama and palisade; $\times 154$.